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# Uterine environment and conceptus development in ruminants

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## Abstract

Interferon tau (IFNT), the pregnancy recognition signal from trophectoderm cells of ruminant conceptuses abrogates the uterine luteolytic mechanism to ensure maintenance of functional corpora lutea for production of progesterone (P4). IFNT acts in concert with P4 to induce expression of genes for transport and/or secretion of histotroph that includes nutrients such as glucose and arginine that activate the mechanistic target of rapamycin (MTOR) nutrient sensing cell signaling pathway to stimulate proliferation, migration, differentiation and translation of mRNAs essential for growth and development of the conceptus. Arginine, leucine, glutamine and glucose increase in the uterine lumen during the peri-implantation period of pregnancy due to increased expression of their transporters by uterine luminal epithelium (LE) and superficial glandular epithelium (sGE) in response to P4 and IFNT. In day 16 ovine conceptus explant cultures, arginine increases GTP cyclohydrolase 1 mRNA, and IFNT, while arginine and glucose increase ornithine decarboxylase, nitric oxide synthase 2, and GCH1. Arginine can be metabolized to nitric oxide (NO) and polyamines which stimulate proliferation of ovine trophectoderm (oTr) cells. Secreted phosphoprotein 1 (SPP1, also known as osteopontin) in uterine histotroph increases focal adhesion assembly as a prerequisite for adhesion and migration of oTr cells through activation and cross-talk between MTOR, MAPK, and myosin II motor pathways. Glucose, arginine, leucine and glutamine stimulate MTOR signaling, proliferation and mRNA translation by oTr cells. Further, glucose and fructose were equivalent in stimulating proliferation and synthesis of hyaluronic acid via the hexosamine pathway in oTr and pig Tr cells. These mechanisms allow select nutrients and SPP1 to act coordinately to affect synthesis of proteins involved in cell signaling affecting conceptus growth, development, and survival during the peri-implantation period of pregnancy.

**Keywords**: amino acids, conceptus, glucose, MTOR, pregnancy, secreted phosphoprotein 1, uterus.

## Conceptus development and pregnancy recognition in sheep

Sheep embryos enter the uterus on day 3, develop to spherical blastocysts, hatch from the zona pellucida and then transform from spherical to tubular and filamentous conceptuses (embryo and associated extra-embryonic membranes) between days 12 and 15 of pregnancy, with extra-embryonic membranes extending into the contralateral uterine horn between days 16 and 20 of pregnancy (Bazer and First, 1983). Elongation of ovine conceptuses is a prerequisite for central implantation involving apposition and adhesion between trophectoderm and uterine luminal epithelium (LE). There is also transient loss of uterine LE that allows intimate contact between conceptus trophectoderm and uterine stromal cells until about day 25 when uterine LE is restored in the intercaruncular endometrium (Guillomot, 1995). All mammalian uteri contain uterine glands that produce or selectively transport a complex array of proteins and other molecules known collectively as histotroph that is required for elongation and development of conceptuses (Spencer and Bazer, 2004). Ewes lacking uterine glands and histotroph fail to exhibit normal estrous cycles or maintain pregnancy beyond about day 14.

In ruminants, the antiluteolytic hormone for pregnancy recognition and maintenance of functional corpora lutea (CL) is interferon tau (IFNT; Bazer *et al*., 2010, 2011). The secretion of IFNT by mononuclear cells of the ovine trophectoderm is developmentally regulated with onset of secretion occurring as large spherical blastocysts transition to tubular and elongated filamentous forms between days 10 and 21 of pregnancy. Ovine IFNT plays a central role in molecular mechanisms that underlie both pregnancy recognition signaling and establishment and maintenance of pregnancy (Bazer *et al*., 2009, 2010, 2011). IFNT silences transcription of estrogen receptor alpha (ESR1) and, therefore, ESR1-dependent expression of the oxytocin receptor (OXTR) gene in both uterine LE and superficial glandular epithelium (sGE), hereafter referred to as LE/sGE. This abrogates development of the endometrial luteolytic mechanism that requires oxytocin-induced release of luteolytic pulses of prostaglandin F2α (PGF) by uterine LE/sGE; however, circulating concentrations of PGF are greater in pregnant than cyclic ewes due to continued expression of prostaglandin endoperoxide synthase 2 (PTGS2; Bazer *et al*., 2010).

In addition to initiating recognition of pregnancy, IFNT regulates the expression of endometrial genes in a cell-specific manner. IFNT directly induces uterine GE

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and stromal cells to express classical interferon stimulated genes (ISGs) that include signal transducer and activator of transcription (STAT1, STAT2) interferon regulatory factors (IRF1, IRF9), interferon-stimulated gene 15 (ISG15), myxovirus resistance 1, mouse, homolog of (MX), 2',5'-oligoadenylate synthetase 1 (OAS), and radical s-adenosyl methionine domain-containing protein 2 (RSAD2). However, these genes are not expressed in uterine LE/sGE because IFNT induces expression of IRF2, a potent repressor of transcription, in uterine LE/sGE (Spencer *et al*., 2008). Thus, uterine LE/sGE lack STAT1, STAT2 and IRF9 required for expression of classical interferon stimulated genes (ISG). Therefore, STAT1-independent cell signaling pathways are responsible for IFNT-stimulation of novel genes by uterine LE/sGE that are critical for implantation and establishment and maintenance of pregnancy. IFNT may induce alternative cell signaling pathways in ovine uterine LE/sGE that include mitogen activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K; Kim *et al*., 2003; Plantanias, 2005).

Progesterone is permissive to the actions of IFNT. The paradox of mammalian pregnancy is that cessation of expression of P4 receptor (PGR) and ESR1 by uterine epithelia is a prerequisite for uterine receptivity to implantation, as well as for expression of novel genes by uterine LE/sGE. Receptors for P4 are absent from ovine uterine LE/sGE after day 13 of the estrous cycle and pregnancy because P4 down-regulates expression of PGR. Therefore both PGR and ESR1 are silenced in uterine LE/sGE during the peri-implantation period in sheep. This is critical since the absence of PGR in uterine LE/sGE is required for P4 to induce genes, some of which are further stimulated by IFNT, that support conceptus growth, development and implantation (Spencer *et al*., 2004a, b; Bazer *et al*., 2010). These novel genes include solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 (SLC7A2), cystatin C (CST3), cathepsin L (CTSL), solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1), hypoxia-inducible factor 1, alpha subunit (HIF2A), and galectin 15 (LGALS15). These genes encode for proteins that are secreted into the uterine lumen, as well as transporters for delivery of select nutrients into the uterine lumen to support conceptus development (Bazer *et al*., 2010, 2011).

Down-regulation of PGR correlates with loss of MUC1 on uterine LE to allow for implantation (Johnson *et al*., 2001; Carson *et al*., 2002). Further, silencing expression of PGR in uterine epithelia allows P4 to act via PGR-positive uterine stromal cells to induce expression of progestamedins, particularly fibroblast growth factor-10 (FGF10) to exert paracrine effects on uterine LE/sGE and conceptus trophectoderm that express receptors for FGF10 (FGFR2IIIb) and HGF (MET; protooncogene MET; Satterfield *et al*., 2008). Importantly, silencing ESR1 expression in uterine LE/sGE by IFNT also prevents the potential for estrogens to induce PGR in uterine LE/sGE, as well as uterine GE. This is critical since the absence of PGR in uterine LE/sGE is required for implantation and expression of genes that are P4-induced, or P4-induced and further stimulated by IFNT, in support of conceptus growth and development (Spencer *et al*., 2004a, b; Bazer *et al*., 2010). Clearly, there is complex temporal (day of pregnancy) and spatial (cell-specific) regulation of expression of P4-induced and novel IFNT-stimulated genes expressed by uterine LE/sGE that is in direct contact with conceptus trophectoderm and considered critical to conceptus development and implantation.

## Select nutrients and MTOR cell signaling in the pregnant uterus

Mammalian cell growth in general, and particularly in cells of the conceptus, is regulated by growth factors and the availability of nutrients (Bazer *et al*., 2012). The MTOR cell signaling pathway plays an important role in regulation of cell growth and metabolism in response to growth factors and nutritional status to affect biological and physiological responses of cells and organs. MTOR is an evolutionarily conserved serine/threonine kinase located downstream of PI3K and AKT1 that controls cell growth and proliferation through activation of ribosomal protein S6 kinase (RPS6K) to phosphorylate RPS6 and ultimately regulate protein synthesis (Hay and Sonenberg, 2004; Wullschleger *et al*., 2006), as well as initiate mRNA translation, ribosome synthesis, expression of metabolism-related genes, autophagy and cytoskeletal reorganization (Kim *et al*., 2002). The MTOR pathway is a "nutrient sensing system" stimulated by molecules that include SPP1, insulin-like growth factor 2 (IGF2), glucose and select amino acids (Nielsen *et al*., 1995; Kimball *et al*., 1999; Martin and Sutherland, 2001; Martin *et al*., 2003; Kim *et al*., 2008) to support blastocyst/conceptus development. Homozygous Frap1 (Mtor) null mice die shortly after implantation due to impaired cell proliferation and hypertrophy in both the embryonic disc and trophoblast (Murakami *et al*., 2004).

In ewes, total recoverable glucose, arginine, leucine, glutamine, as well as other amino acids, and glutathione, calcium and sodium are more abundant in uterine fluids of pregnant than cyclic ewes between days 10 and 16 after onset of estrus (Gao *et al*., 2009f). Therefore, we determined tissue and cell-specific gene expression for select facilitative (SLC2A1, SLC2A3 and SLC2A4) and sodium-dependent glucose transporters (SLC5A1 and SLC5A11; Gao *et al*., 2009a), cationic amino acid transporters (SLC7A1, SLC7A2 and SLC7A3; Gao *et al*., 2009b), neutral (SLC1A4, SLC1A5, SLC3A1, SLC6A14, SLC6A19, SLC7A8, SLC38A3, SLC38A6, SLC7A8 and SLC43A2), and acidic amino acid transporters (SLC1A1, SLC1A2 and

SLC1A3; Gao *et al*., 2009c). Among these genes, SLC2A3 and SLC7A6 were detectable only in trophectoderm and endoderm of conceptuses. The abundance of mRNAs for SLC2A1, SLC2A4, SLC5A1, SLC5A11, SLC7A1, SLC7A2, SLC1A4, SLC1A5, SLC43A2 and SLC1A3 in ovine uterine endometria varies according to day of the estrous cycle and early pregnancy. Expression of mRNAs for SLC1A5, SLC2A1, SLC5A11 and SLC7A1 in endometria was induced by P4 and further stimulated by IFNT. Collectively, results of our studies indicate the presence of the mRNAs and proteins associated with both MTORC1 and MTORC2 cell signaling in the ovine uterus and conceptus, as well as nutrient transporters that account for increased transport of arginine, leucine, glutamine and glucose into the uterine lumen and conceptuses.

The MTOR cell signaling pathway is a prominent component of the peri-implantation intra-uterine environment in sheep. Progesterone and IFNT stimulate expression of ras homolog enriched in brain (RHEB) and eukaryotic translation initiation factor 4e-binding protein 1 (EIF4EBP1) in ovine uterine endometria, resulting in increased abundance of mRNAs for rapamycin-insensitive companion of MTOR (RICTOR), regulatory associated protein of MTOR (RAPTOR), RHEB and EIF4EBP1, as well as RHEB protein which is coordinate with rapid growth and development of ovine conceptuses during the peri-implantation period (Gao *et al*., 2009e). The abundance of MTOR associated protein LST8 (LST8), mitogen-activated protein kinase-associated protein 1 (MAPKAP1), RHEB and EIF4EBP1 mRNAs in ovine conceptuses during early pregnancy increases coincident with their growth and development (Gao *et al*., 2009e), and MTORC1 is abundant in the cytoplasm and phosphorylated MTOR is particularly abundant in the nuclei of trophectoderm and endoderm cells (Kim *et al*., 2011a). Our recent research with pregnant and cyclic ewes has focused on the presence of select nutrients, amino acids and glucose, in uterine histotroph that affect MTOR cell signaling, as well as expression of transporters for glucose and arginine and enzymes affecting metabolism of arginine.

The effects of the estrous cycle, pregnancy, P4 and IFNT on expression of nitric oxide synthase (NOS1, NOS2A and NOS3), GTP cyclohydrolase (GCH1), and ornithine decarboxylase 1 (ODC1) have been examined in sheep conceptuses and uteri. Both NOS1 and ODC1 are expressed by uterine LE/sGE while NOS3 is most abundant in conceptus trophectoderm and endoderm (Gao *et al*., 2009d). Expression of GCH1 for synthesis of tetrahydrobiopterin, the cofactor for all NOS isoforms for NO production, as well as ODC1 and NOS1 is more abundant in conceptuses than endometrial cells (Gao *et al*., 2009d). P4 stimulates expression of NOS1 and GCH1, while IFNT inhibits expression of NOS1 (Gao *et al*., 2009d). Therefore, key molecules for metabolism of arginine (NOS2, NOS3), and ornithine (ODC1) are present to account for production of NO and polyamines that affect growth and development of the conceptus.

## Pathways for arginine-mediated effects on proliferation and migration of ovine trophectoderm cells

Arginine is highly stimulatory to proliferation, migration and protein synthesis in oTr cells (Kim *et al*., 2011a, b). Studies of pathways whereby arginine mediates its effects in oTr cells revealed that arginine: 1) increases phosphorylation of RPS6K in a dose-dependent manner with maximum effects at 0.2 mM; 2) increases phosphorylated forms of AKT1, RPS6K and RPS6 over basal levels within 15 min and the effect is maintained to 60 min; 3) increases nuclear phosphorylated RPS6K and cytoplasmic phosphorylated RPS6 within 30 min; and 4) stimulates proliferation and migration of trophectoderm cells (Kim *et al*., 2011a). Further, phosphorylation of RPS6K and RPS6 is blocked by inhibitors of both PI3K and MTOR cell signaling. L-arginine, but not D-arginine, activates MTOR cell signaling via phosphorylation of RPS6K and RPS6 (Kim *et al*., 2011b).

The effects of arginine on proliferation of oTr cells are due in part to its metabolism to NO via NOS1/NOS2 and due to its metabolism by arginase to ornithine which is converted by ODC1 to polyamines (putrescine, spermidine and spermine; Kim *et al*., 2011c). Two NO donors, *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) and diethylenetriamine NONOate (DETA), increased proliferation of oTr cells as did putrescine, a polyamine. Both L-NAME (NOS inhibitor to reduce NO) and nor-NOHA (arginase inhibitor to block synthesis of putrescine) decreased oTr cell proliferation. Therefore, both NO and polyamines can stimulate proliferation and migration of oTr cells, but neither of the inhibitors of arginine metabolism fully suppress effects of arginine. Arginine may act via other cell signaling pathways, such as Rac activation (Hernandes-Negrete *et al*., 2007), to stimulate cell proliferation and migration. Arginine can also activate mitogen-activated protein kinase/extracellular-signal-regulated kinase MAPK/ERK) signaling, but the mechanism(s) whereby arginine acts on mammalian cells to activate MTORC1/MTORC2 and/or MAPK/ERK is unknown (Yan and Lamb, 2011).

## Response of ovine conceptus explant cultures to select nutrients

Due to the possibility that the phenotype of cultured oTr cells differs from that of cultured conceptuses, effects of select nutrients were evaluated using day 16 sheep conceptus explant cultures. The culture medium was supplemented with arginine, leucine, glutamine or glucose to assess their differential

effects on expression of mRNAs and total and phosphorylated forms of proteins in the MTOR cell signaling pathway, as well as translation of mRNAs for key proteins associated with conceptus development and pregnancy recognition signaling. The abundance of transcripts for MTOR, RPS6K, RPS6, EIF4EBP1, NOS1 (neuronal), NOS2 (inducible), NOS3 (endothelial), GCH1, ODC, and IFNT in conceptuses in control and in nutrient-supplemented medium was determined (Kim *et al*., 2011c). Only conceptuses treated with arginine had increased expression of GCH1 mRNA. However, compared with the control conceptus explant cultures, arginine increased total and phosphorylated forms of MTOR, RPS6K, RPS6 and EIF4EBP1. Arginine also increased IFNT, ODC1, NOS2 and NOS3. Leucine increased total and phosphorylated forms of MTOR, RPS6K, RPS6 and EIF4EBP1, but did not affect the abundance of IFNT, ODC1, NOS2, NOS3 and GCH1 in conceptuses. Glucose stimulated the abundance of total and phosphorylated forms of the MTOR cell signaling pathway proteins, as well as ODC1, NOS2 and GCH1. Glutamine increased total and phosphorylated forms of RPS6, RPS6K, and EIF4EBP1, but only nonphosphorylated MTOR. Further, glutamine did not increase expression of ODC1, NOS2 and GCH1, but did increase the abundance of NOS3 (Kim *et al*., 2011c). These results indicate that arginine-induced cell signaling via MTORC1 stimulates secretion of IFNT. With P4 being permissive, IFNT increases expression of cationic amino acid transporters to deliver more arginine into the uterine lumen to enhance conceptus development and secretion of IFNT.

### Exogenous P4 advances elongation of ovine conceptuses and transport of select nutrients into the uterine lumen

Growth and development of the conceptus is dependent on uterine LE/sGE and middle-to-deep GE to produce histotroph in response to P4, with many of these effects of P4 likely mediated via progestamedins and IFNT (Spencer and Bazer, 2004; Bazer *et al*., 2011) as well as prostaglandins (Dorniak *et al*., 2011). A delay in the increase in circulating concentrations of P4 during metestrus and diestrus is associated with retarded conceptus development and reduced or delayed secretion of IFNT on day 17 in cattle (Garret *et al*., 1988; Kleeman *et al*., 1994; Mann and Laming, 2001; Mann *et al*., 2006). This adversely affects secretion of IFNT which increases coordinately with elongation of the conceptus to the filamentous form up to days 15 to 16 of pregnancy (Spencer and Bazer, 2004).

An ovine model of early administration of exogenous P4 at 36 h after onset of estrus, i.e., about 6 h post-ovulation, has been used to advance conceptus development and IFNT secretion in both sheep and cattle. Using this model it was found that P4 accelerated conceptus development and also advanced expression of uterine genes that favor survival, growth and development of the conceptus (Satterfield *et al*., 2006, 2007, 2008; Carter *et al*., 2008). An early increase in circulating concentrations of P4: 1) advances the time of down-regulation of PGR in uterine epithelia and onset of secretion and abundance of IFNT in uterine flushings; 2) increases abundance of secreted proteins such as galectin 15 (LGALS15), cathepsin L (CTSL), gastrin releasing protein (GRP), stanniocalcin, and insulin like growth factor binding protein 1 (IGFBP1) by uterine LE/sGE (Song *et al*., 2005, 2006, 2008; Gray *et al*., 2006; Satterfield *et al*., 2006, 2008); 3) increases expression of FGF10 and, to a lesser extent, MET mRNA suggesting that FGF10 is an important uterine stromal cell-derived progestamedin stimulated by P4 (Satterfield *et al*., 2008); 4) increases MET mRNA that increases responsiveness of uterine LE/sGE to HGF to enhance conceptus development because FGFR2IIIb and MET are expressed by both uterine epithelia and trophectoderm (Chen *et al*., 2000a, b; Satterfield *et al*., 2008); 5) decreases tight-junction associated proteins in uterine LE that may facilitate paracellular trafficking and/or transport of stromal and serum-derived molecules (Satterfield *et al*., 2007); 6) increases total recoverable glucose, aspartic acid, asparagine, serine, and alanine, glutamine and beta-alanine, citrulline, arginine, and lysine in the uterine lumen on day 9 (Satterfield *et al*., 2010); 7) increases steady-state levels of SLC2A1 and SLC5A1 mRNAs and proteins in uterine LE/sGE for glucose transport; and 8) increases steady-state levels of SLC7A2 mRNA in uterine LE/sGE for transport of cationic amino acids, particularly arginine (Satterfield *et al*., 2010).

In cows, a 3-fold increase in circulating concentrations of P4 increased recovery rates of blastocysts and induced a 2.3-fold increase in blastocyst size on day 13 of pregnancy (Lonergan *et al*., 2007; Carter *et al*., 2008), as well as increasing the frequency of elongated conceptuses on day 16 of pregnancy (Carter *et al*., 2008). These effects of P4 on conceptus development were found to be mediated via the endometrium (Clemente *et al*., 2009). Early P4 treatment in cattle advances down-regulation of PGR (Okuma *et al*., 2010) and increases expression of genes associated with nutrient transport such as SLC5A1 (glucose transporter), nutrient availability such as DGAT2 (diacylglycerol–o-acetyltransferse for synthesis of triglycerides), MSTN (myostatin or growth/differentiation factor 8) that affects embryonic development and muscle mass, FABP (fatty acid binding protein) and CRYGS (crystalline gamma-s for development of the lens in the eye; Forde *et al*., 2009). Forde *et al*. (2010) also found increases in expression of CTGF (connective tissue growth factor), LPL (lipoprotein lipase), and SLC5A1 (sodium/glucose co-transporter) mRNAs in response to high concentrations of P4 in cows. These results indicate that P4 modifies the uterine environment by modifying the composition of histotroph to advance

conceptus development (Forde *et al*., 2011).

### *In vivo* effects of arginine on successful outcomes of pregnancy

Recognition of the importance of arginine in survival and growth of the conceptus led to development of treatment protocols to determine whether arginine increases successful pregnancy outcomes in animals and humans (see Wu *et al*., 2009). Dietary supplementation with Arg-HCl increases fetal survival in gilts (Mateo *et al*., 2007), as well as embryonic survival and litter size in rats (Zeng *et al*., 2008). In ewe models of both undernutrition-induced and naturally-occurring intrauterine growth retardation, intravenous administration of Arg-HCl enhanced fetal growth (Lassala *et al*., 2009, 2011). Also, in women with intrauterine growth retardation of their fetus at week 33 of gestation, daily intravenous infusions of arginine increased birth weight at term (Xiao and Li, 2005).

### MTOR cell signaling activated by SPP1 and integrins

SPP1 is a matricellular protein that is expressed and secreted by the middle-to-deep GE by day 13 of pregnancy in response to P4 (Johnson *et al*., 1999a, b, 2003). Large amounts of SPP1 protein binds to both the conceptus trophectoderm and uterine LE throughout pregnancy.

Attachment and migration of trophectoderm cells are hallmarks of conceptus development and implantation in mammals. SPP1 in the uterus binds integrins on conceptus trophectoderm and uterine LE to affect cell-cell and cell-matrix interactions (Johnson *et al*., 2003). SPP1 induces motility in human trophoblast cells through MTOR signaling (Al-Shami *et al*., 2005) and rapamycin inhibits F-actin reorganization and phosphorylation of focal adhesion proteins stimulated by IGF1 such as focal adhesion kinase (FAK; Liu *et al*., 2008). These results indicate involvement of SPP1-induced MTOR complex signaling in key events of pregnancy. Therefore, we identified relationships and crosstalk between multiple membrane and intracellular cell signaling cascades activated by SPP1, including MTOR, and integrin binding to oTr cells that control proliferation, migration, attachment and adhesion in conceptuses during the peri-implantation period of pregnancy (Kim *et al*., 2010). SPP1 binds ITGAV:ITGB3 and possibly ITGA5:ITGB1 integrin heterodimers to induce focal adhesion assembly, a prerequisite for adhesion and migration of trophectoderm cells, through activation of: 1) P70S6K via crosstalk between MTOR and MAPK pathways; 2) MTOR, PI3K, MAPK3/MAPK1 (ERK1/2) and MAPK14 (P38) signaling to stimulate trophectoderm cell migration; and 3) focal adhesion assembly and myosin II motor activity to induce migration of trophectoderm cells (Kim *et al*., 2010). These cell signaling pathways, act in concert to mediate adhesion, migration and cytoskeletal remodeling of trophectoderm cells essential for expansion and elongation of conceptuses and attachment to uterine LE for implantation.

### Key roles for fructose in development of ungulate conceptuses

In ungulates (e.g., pigs and sheep), and cetaceans (e.g., whales) glucose is transported into the uterus and, in pregnant females, glucose not utilized for energy metabolism is converted to fructose (Bacon and Bell, 1948). Glucose is an energy source for proliferation and growth of mammalian cells, but the role of fructose is unclear although it is the most abundant hexose sugar in fetal blood and fluids of ungulate and cetacean species of mammals. The role of fructose in conceptus growth and development has been ignored since it is not metabolized via the glycolytic pathway or the Krebs cycle as an energy source. We hypothesized that fructose is metabolized via multiple metabolic pathways critical to pregnancy and metabolism in multiple organ systems of the fetus. Our results from studies using a porcine trophectoderm cell line indicate that fructose and glucose are equivalent in being metabolized via the MTOR nutrient sensing pathway to increase cell proliferation and mRNA translation and via the hexosamine pathway to potentially stimulate MTOR cell signaling and production of glycosaminoglycans that are critical to growth and development of the conceptus (J. Kim, G. Song, G. Wu and F.W. Bazer, unpublished results).

### Summary

This review focuses on the critical role of uterine endometrium, i.e., LE/sGE and middle-to-deep GE, and their secretions for normal conceptus development. It has provided evidence that SPP1 and select nutrients, such as arginine, activate the MTOR nutrient sensing pathway and focal adhesion assembly necessary for growth, development and differentiation of the trophectoderm during conceptus development, implantation and establishment and maintenance of pregnancy. Early exogenous P4 accelerates conceptus growth and development, increases total amounts of select nutrients, such as arginine and glucose, in the uterine lumen, induces early down-regulation of PGR in uterine epithelia, and advances onset of secretion of IFNT for pregnancy recognition signaling. Thus, humans and animals that experience an early post-ovulation increase in circulating P4 may have an increased rate of reproductive success. Advances in transport of select nutrients, particularly arginine and glucose, and secretion of various proteins, including SPP1, by uterine LE/sGE and GE induce conceptus

development. Finally, we have demonstrated that glucose and fructose are equivalent in their actions to stimulate MTOR cell signaling, cell proliferation and production of glycosaminoglycans critical to growth and development of the conceptus. Future research will determine whether P4 supplementation translates into increased reproductive success and whether dietary supplementation with select nutrients such as arginine increase successful pregnancy outcomes in animals and humans.

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## Conflicts of interest

The authors declare that they have no conflicts of interest.

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